Interactome analysis of devS protein involved in persistence of *Mycobacterium tuberculosis* and design of inhibitor against its interacting persister protein: An approach to inhibit protein –protein interaction

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Abstract

Background: *Mycobacterium tuberculosis* has been a potential threat for humans for ages. Its invulnerability to various drugs and persistency has emerged as a stumbling block in eradicating the pathogenecity of the bacteria. A protein-protein interaction network of redox sensor histidine kinase response regulator (devS), a member of the two- component regulatory system devR/devS is known to be involved in onset of the dormancy response acting as a redox sensor was studied.

Methods: An interactome level analysis of devS with other proteins involved is essential to gain insights into the proteins involvement in persistence of tuberculosis. Folding pattern of the proteins involved in the interaction was analyzed and molecular docking was performed to understand the protein-ligand interaction.

Result: DevS protein directly interacts with high confidence with transcriptional regulatory protein (devR) protein forming a two-component system, probable transcriptional regulatory (narL) protein and a universal stress protein (MT3220). Hypoxia sensor histidine kinase response regulator dosT (MT2086) interact with the two-component regulatory system devR/devS involved in dormancy and is structurally aligned with devS protein. The folding patterns of devS, MT2086 and MT0867 are similar but at a different folding rate.

Conslusion: DevS is shown to interact with devR protein with high confidence, which is involved in the two-component system. A better interaction is seen with piperine, berberine and allin with all the four target proteins.

Keywords: Mycobacterium tuberculosis, persister proteins, interactome.

1. Introduction

Among all ancient diseases, tuberculosis has been a major threat to humanity caused by *Mycobacterium tuberculosis*. The pathogen is capable of causing both acute disease process and an asymptomatic latent infection (Parrish et al., 1998). In 2007, there were an estimated 13.7 million chronic active cases and by 2010 there were 8.8 million new cases of TB diagnosed, and 1.45 million deaths, most of these occurring in developing countries. Of these 1.45 million deaths, about 0.35 million occur in those co-infected with HIV (WHO report, 2009; WHO report 2011). Due to the extensive period of treatment, patients fail to complete the therapy leading to the emergence of drug resistance-multi drug resistance (MDR), extensive drug resistance (XDR) and total drug resistance (TDR) in tuberculosis. The current scenario of drug resistance and with the recent emergence of total drug resistance of tuberculosis in India (Udwadi et al., 2012), it has become an alarming threat to control the disease globally (Udwadi et al., 2012).

The pathogen has a distinctive capability of becoming dormant for a long period of time and evades the host response and is the characteristic feature of the pathogen to reside inside the mononuclear phagocytes by exhibiting specific cellular equilibrium for the phagocytes interfering with the human immune system (Ellner, 1997; Hingley-Wilson et al., 2003; Russell, 2001). The bacteria reside inside the alveolar macrophage vesicular compartment, replicate and migrate to the granuloma where the microbe can persist for years (Armstrong and Hart, 1971; Noss et al., 2002). Two conditions commonly related with latent TB are reduced oxygen tension and nitric oxide (NO) exposure (Wayne and Sohaskey, 2001; Nathan and Shiloh, 2000). Mycobacterium requires oxygen for growth but surprisingly it can survive without oxygen for long periods of time (Wayne and Lin, 1982). Hypoxia, nitric oxide and nutrient starvation are some of the conditions, which are believed to be associated with initiation and maintenance of tuberculosis dormancy (Chauhan et al., 2011). Redox sensor histidine kinase response regulator (devS) which is a member of the two-component regulatory system devR/devS is rapidly up-regulated in response to reduced oxygen tension or NO (Voskuil et al., 2003; Sherman et al., 2001). DevS is known to involve in onset of the dormancy response (Roberts et al., 2004) and is induced in response to reduced oxygen

tension (hypoxia), low levels of nitric oxide (NO) and carbon monoxide (CO) (Voskuil et al., 2003; Sherman et al., 2001). In the absence of devS, there is no change in gene induction following hypoxia, or exposure to NO or CO and show no response (Shiloh et al., 2008).

The present study is aimed to understand the protein interactions involved in persistency and formulating inhibitors against the enzymes involved in the network, which would prevent the onset of dormancy of the microbe. It would be a novel approach to find a common drug that would target proteins involved in dormancy, in a single protein interacting network.

2. Methodology

A dataset of 60 natural active antimicrobial compounds obtained from different natural resources were retrieved from Pubchem (http://pubchem.ncbi.nlm.nih.gov/) and Drug Bank databases (http://www.drugbank.ca/). Molecular properties and prediction of bioactivity of all the compounds was calculated using Molinspiration server (http://www.molinspiration.com/cgi-bin/properties) and Osiris Property Explorer (http://www.organic-chemistry.org/prog/peo/) for various drug relevant properties like mutagenic, tumorigenic, irritant and reproductive effect of the compounds following Lipinski Rule of five (Lipinski et al., 1997). An interactome level analysis of DevS known to involve in dormancy response was performed to find out the proteins interacting with it. For this proteome-scale interaction network in *Mycobacterium tuberculosis*, STRING database, was used with 'high-confidence' criteria (Szklarczyk et al., 2011).

Structure analysis of the interacting proteins was performed. The three-dimensional structure of the Redox sensor histidine kinase response regulator (devS), Transcriptional regulatory protein devR (devR), Probable transcriptional regulatory protein (narL) and Hypoxia sensor histidine kinase response regulator dosT (MT2086) proteins was retrieved from the Protein Data Bank with PDB Ids 2W3F, 3C3W, 3EUL and 2VZW respectively. Active site of narL protein was performed using CastP Server. Folding patterns of the proteins were analyzed using Phyre and K-fold which identifies the fold and domain in the proteins and predicts the folding kinetic order and rate (Capriotti and Casadio, 1997). To understand the mechanism of ligand binding and interaction, molecular docking of the proteins was performed with the filtered compounds using Gold suite 5.0.1.

Pharmacophore modeling was performed using MolSign module of Vlife MDS 4.2 software. The best compound that showed good docking energy and hydrogen bond interaction with the proteins was considered for pharmacophore modeling. The minimum number of pharmacophoric features generated for an alignment was taken as three and the tolerance for the distance separating two features was kept at 10 Å.

3. Results and Discussion

Ligands with antimicrobial activity were analyzed for their molecular property and drug-like ability. Of the 60 natural compounds that were retrieved only 44 compounds followed Lipinski Rule of Five and were further considered for toxicity test, out of which only 14 compounds were found to be non – toxic with no risk and no side effect (Table 1). Structures of the compounds for study are shown in figure 1.

Fig.1. *Structure of compounds selected for study*

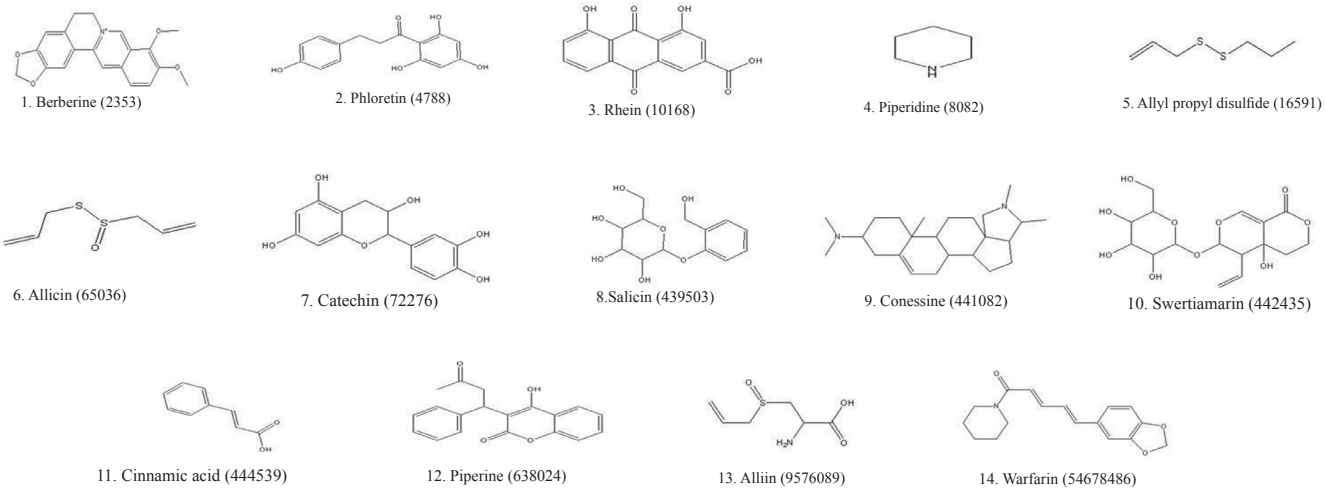


Table 1. *Toxicity risk of selected compounds as predicted by Osiris*

	Compound	Mutagenic	Tumorigenic	Irritant	Reproductive effect	cLogP	Solubility	Molecular weight	Drug-likeness	Drug Score
1.	Warfarin 54678486	No risk	No risk	No risk	No risk	2.93	-3.54	310	1.85	0.76
2.	Alliin 9576089	No risk	No risk	No risk	No risk	-0.21	-1.22	161	-8.97	0.49
3.	Piperin 638024	No risk	No risk	No risk	No risk	4.72	-4.92	298	-4.1	0.29
4.	Cinnamic acid 444539	No risk	No risk	No risk	No risk	1.63	-2.0	148	-1.1	0.36
5.	Swertiamarin 442435	No Risk	No Risk	No Risk	No Risk	-1.91	-0.86	390	-5.83	0.44
6.	Conessine 441082	No Risk	No Risk	No Risk	No Risk	3.91	-3.55	356	6.07	0.73
7.	Salicin 439503	No Risk	No Risk	No Risk	No Risk	-1.05	-1.09	286	-4.88	0.48
8.	Catechin 72276	No Risk	No Risk	No Risk	No Risk	1.88	-1.76	290	1.92	0.87
9.	Allicin 65036	No Risk	No Risk	No Risk	No Risk	0.88	-1.22	162	-6.13	0.49
10.	Allyl propyl disulfide 16591	No risk	No risk	No risk	No risk	0.89	-2.77	148	-5.03	0.47
11.	Rhein 10168	No risk	No risk	No risk	No risk	2.44	-4.15	284	0.18	0.61
12.	Piperidine 8082	No risk	No risk	No risk	No risk	1.04	-1.13	85	-2.89	0.51
13.	Phloretin 4788	No risk	No risk	No risk	No risk	2.45	-2.52	274	-0.56	0.61
14.	Berberine 2353	No risk	No risk	No risk	No risk	3.38	-3.45	337	2.77	0.76

Protein-protein interaction of devS protein with other proteins of *Mycobacterium tuberculosis* have been constructed showing three proteins that is devR, narL and MT3220 to be interacting directly and two proteins-putative uncharacterized protein (MT0867) and MT2086 shows an indirect interaction based on the different evidences which are gene fusion, co-occurrence, co-expression, experimental and databases (Kyoto Encyclopedia of Genes and Genomes) (Figure 2). DevS protein directly interacts with high confidence (0.700 score) with transcriptional regulatory protein (devR) protein forming a two-component system involve in persistency and hypoxic condition of the tubercle bacilli, probable transcriptional regulatory (narL) protein which is a member of the two-component regulatory system NarS/NarL and a universal stress protein (MT3220), also involved in dormancy response. Hypoxia sensor histidine kinase response regulator dosT (MT2086) interact with the two-component regulatory system devR/devS involved in dormancy and is structurally aligned with devS protein. The co-expression evidence in *Mycobacterium tuberculosis* and with other microbial species is shown in Figure 3. It is hoped that these protein-protein interaction will give new insight into the latent or dormant phase of infection.

Fig.2. *Protein-protein interaction of devS protein with other Mycobacterium tuberculosis proteins.*

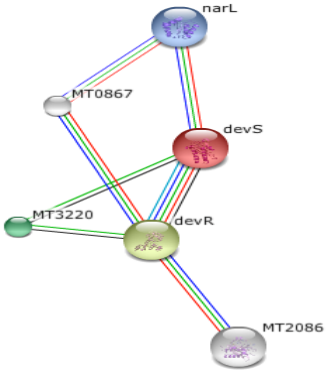
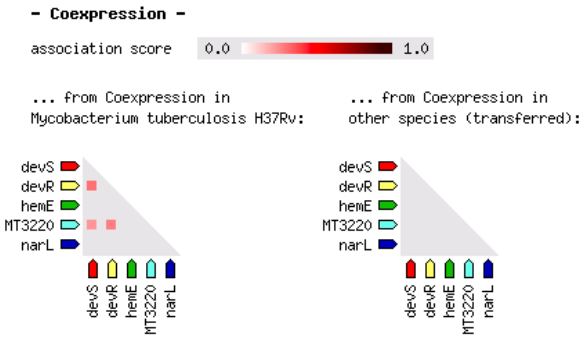
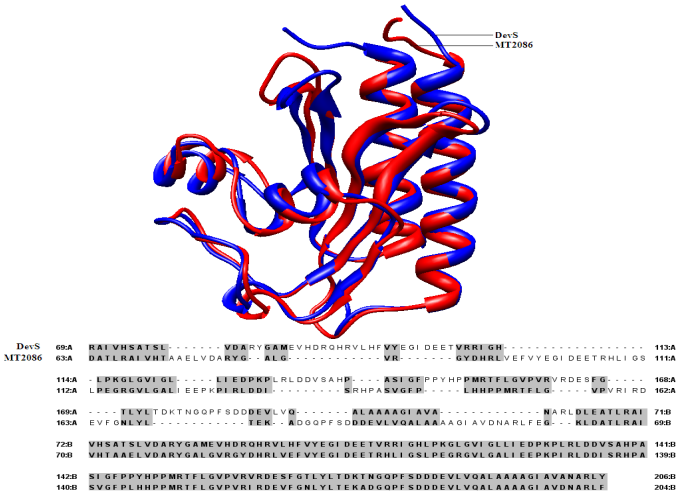


Fig.3. *Co-expression evidence for interaction*



Structural alignment of the proteins shows that devS protein is aligns with MT2086 (Figure 4) indicating that MT2086 have the same function as devS protein, which is involved in onset of the dormancy response.

Fig.4. *Structure and sequence alignment of devS and MT2086 proteins*



Folding pattern of the proteins is different from each other except for devS, MT2086 and MT0867 proteins where same folding pattern at a different folding rate has been observed (Table 2). The folding mechanism and rate for MT3220 and MT0867 was not determined since structures are not available. The secondary structures of the proteins are predicted with very few disordered sequence (Figure 5). The compounds passing the molecular properties and drug-like ability were then docked with devR, narL, MT2086 and devS proteins and it shows good binding with target proteins. Piperine has a better docking result with the proteins having a docking score of 55.4105 with one H-bond, 44.8690 with three H-bonds and 52.9538 with one H-bond with devS, devR and MT2086 proteins, respectively (Figure 6). Swertiamarin shows a good interaction with the proteins and the best interaction is observed with devS (docking score-49.6036 with two H-bonds). Berberine and allicin also shows a good interaction with devR (docking score- 47.4067 and 36.6841 with three H-bonds) and MT2086 (docking score-57.6833 and 38.3740 with one H-bond). Allin shows a good interaction with the devR (docking score-44.8690 with three H-bonds), devS (docking score-38.7245 with two H-bonds) and MT2086 (docking score-38.6187 with three H-bonds) (Table 3). The interactions are electrostatic in nature affecting the protein structure and stability with the ligand molecules inside the cavity (Figure 7). The oxygen atom of piperine and berberine mostly contributes to the interactions with the target proteins indicating that it may be the key atom required to inhibit the proteins.

Table 2. *Folding patterns and rate of folding for the different proteins*

Protein name	Folding pattern	Folding mechanism		Folding rate	
		CO[6]	States RI	CO[0]	Log[Kf]
devS	Transferase	0.321	MS 4	0.217	0.96
devR	Transcription	0.233	MS 9	0.150	0.83
narL	Transduction protein	0.286	MS 6	0.180	1.24
MT2086	Transferase	0.328	MS 3	0.222	0.93

Where, CO [W]: Contact Order calculated using a sequence separation > W

RI: Reliability Index

States: Kinetic Mechanisms of the Protein Folding

TS: Two-State

MS: Multiple-State

Log [Kf]: Logarithm of the Folding Rate

Table 3. *Docking score of the compounds with the target proteins*

Ligand Name	Docking Score and hydrogen bonding to target proteins			
	DevS	DevR	NarL	MT2086
Warfarin	47.5808 Tyr171...O (2.736)	47.5247 Arg67...O(2.725) Asp68...O(2.733) Arg113...O(2.724) Arg113...O(2.700) Ser162...O(2.999)	-48.0563	48.4819
Allin	38.7245 Tyr83...N1(2.649) Gly84...N1(3.073) Gly84...O1(2.416) Leu172...O2(2.960)	36.2464 Asp59...O1(2.881) Asp172...N1(2.990)	27.1258	38.6187 Glu99...O2(2.966) Glu99...O2(2.764) Ile101...O3(3.040)
Piperine	55.4105 Gly84...O(2.482)	44.8690 Arg155...O(3.036) Glu163...O(2.922) Glu163...O(2.661)	4.0193 Leu110...O(2.519) Ser113...O(2.152)	52.9538 Gly82...O(2.627)
Cinnamic acid	36.1886	34.7644 Asp68...O(2.752)	21.2471	34.1653 Tyr169...O(2.515) Thr171...O(2.465) Thr171...O(2.530)
Swertiamarin	49.6036 Gly85...O (2.272) His419...O (2.834)	44.4152 Arg67...O(2.914) Arg67O(2.338) Arg67...O(2.820) Arg67...O(2.808) Arg67...O(2.752) Arg67...O(2.766) Arg67...O(2.741) Asp68...O(2.311) Asp68...O(2.297) Arg113...O(3.044) Ser162...O(2.953) Glu163...O(2.852) Glu163...O(2.281)	-63.8757 Gly14...O(2.477) Asp15...O(2.691) Phe20...O(3.007) Arg21...O(2.373) Leu59...O(3.000) Leu60...O(2.703) Asp61...O(2.018) Ser113...O(2.439) Ser113...O(2.327)	45.1339 Gly82...O(2.813) Gly82...O(2.668) Ala83...O(2.730) His147...O(2.704)
Conessine	52.0418	37.0704	-396.544	51.4828
Salicin	47.9594 Gly84...O (3.009) Ala85...O(2.629) Glu101...O(2.758) Tyr171...O(2.627)	40.5930 Arg67...O(3.032) Met109...O(2.904) Thr156...O(2.639)	0.5620 Gly14...O(2.531) Gly14...O(2.334) Gly14...O(2.253) Asp15...O(2.285) Leu59...O(2.004)	45.7858 Glu99...O(2.753) Glu99...O(2.522)
Catechin	47.7935 Gly84...O(2.926) Gly84...O(2.536) Glu101...O(2.145) Glu101...O(3.063) Ile103...O(2.664) Val108...O(2.691) His113...O(2.309) His149...O(3.033)	45.5247 Arg67...O(2.665) Arg113...O(2.959) Asp152...O(2.537) Arg155...O(2.777) Gly159...O(2.753) Ser162...O(3.002) Glu163...O(2.495)	-33.2165 Gly14...O(2.351) Asp15...O(2.976) Leu19...O(2.323) Lys111...O(2.003) Asp112...O(3.064) Ser113...O(2.264) Ser113...O(2.099)	48.6453 Glu99...O(2.232) Gly110...O(3.017) Ser111...O(2.978) Ser111...O(2.504)
Allicin	39.5777	36.6841 Glu64...O(2.727 Arg173...O(2.939) Arg173...O(2.664)	29.4923 Leu60...O(2.865) Asp61...O(3.008)	38.3740
Allyl propyl disulfide	39.1194	36.9820	28.5008	37.9815

Rhein	47.0729 Tyr83...O(2.301) Gly84...O(2.786) Gly84...O(2.425) Glu101...O(2.521)	44.8762 Asp152...O(2.658) Thr156...O(2.928)	-28.6523 Gly14...O(2.238) Leu59...O(2.586) Leu60...O(2.970) Leu60...O(2.752) Asp61...O(2.433) Leu87...O(2.781) Ile88...O(2.781) Ser113...O(2.375)	48.2195 Gly82...O(2.824) Glu99...O(2.410)
Piperidine	25.7290 Ser142...N(2.749)	23.0823 Glu64...N(2.950)	21.6893 Gly14...N(2.807) Asp61...N(2.903)	24.5277 Arg106...N(2.529)
Phloretin	47.0179 His113...O(2.949) His149...O(2.656) Leu172...O(2.932)	42.0844 Ala37...O(2.396) Asp68...O(2.871) Arg72...O(3.028) Gly164...O(2.636) Gln169...O(2.454) Asp172...O(2.373) Arg173...O(3.040)	-1.9042 Gly14...O(2.647) Gly14...O(2.274) Asp15...O(2.702) Lys111...O(1.860) Ser113...O(2.684) Ser113...O(2.594)	49.9171 Gly82...O(2.785) Gly110...O(2.917) Leu112...O(2.840)
Berberine	58.5166	47.4067 Asp68...O(2.354) Thr156...O(2.884) Thr156...O(2.595)	-147.7413	57.6833 Gly82...O(2.740)

Fig.5. *Secondary structure and disorder of the proteins predicted*

Fig.6. *Molecular interactions of (a) devR with piperine and (b) devS protein with allin*

a *b*

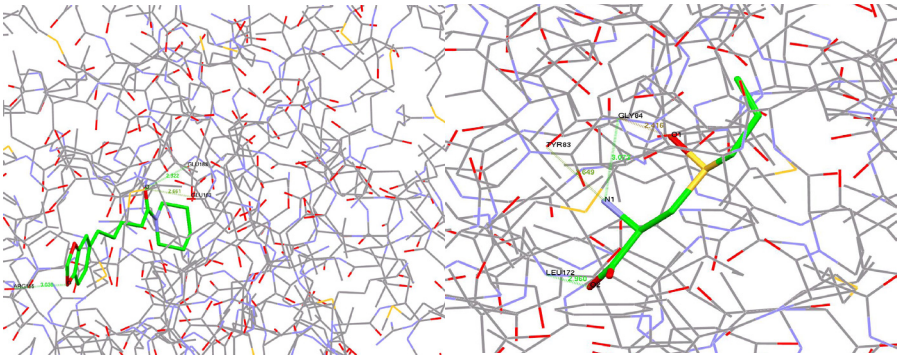
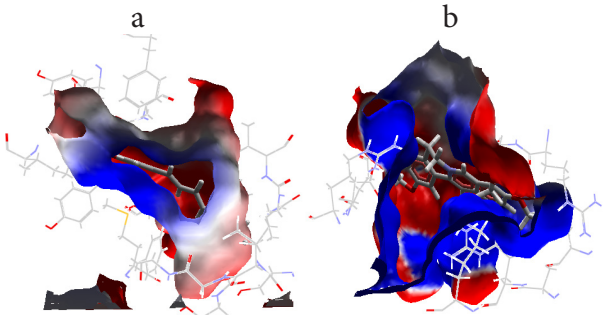


Fig.7. *Electrostatic interactions of (a) piperine and (b) berberine well inside the cavity of the target protein*



The pharmacophore models were validated by comparing with the characteristic feature of the models generated using structures of compounds best docked with the proteins. The oxygen atom, which acts as a hydrogen bond acceptor, is responsible for the hydrogen bond interactions with the proteins. Figure 8, shows the pharmacophore models of the molecules generated. The aliphatic carbon and the aromatic carbon shows to have a pharmacophoric feature but shows no interaction involved with any proteins.

Fig.8. *Pharmacophore models of the piperine and berberine*

4. Conclusion

Based on the findings, the devS protein shows a very high confidence interaction with devR, narL, MT3220, MT2086 and MT0867 proteins that are also involved in dormancy response. Folding of protein differs but devS, MT2086 and MT0867 shows a similar pattern and docking results shows that piperine is a common compound that binds and interacts with the target proteins with high docking score and maximum number of hydrogen bonds. These compounds- piperine, berberine and allin identified, thus holds promise for design of new anti-tuberculosis drugs and can be further validated and their function assessed in vivo.

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